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[http://www.biochemistry.unimelb.edu.au/research/res\\_hill.html](http://www.biochemistry.unimelb.edu.au/research/res_hill.html)

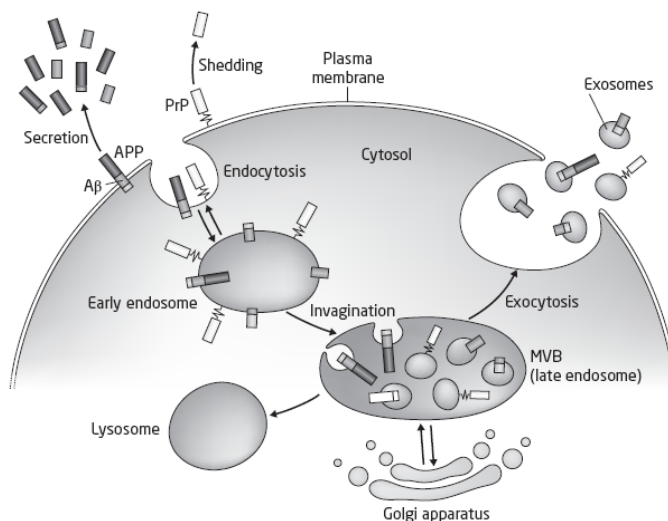
## Processing of proteins and RNA in neurodegenerative diseases

**Prion diseases** such as Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE or 'mad-cow' disease) in cattle are invariably fatal neurodegenerative diseases. Prions differ from conventional infectious agents in being highly resistant to treatments that destroy the nucleic acids found in bacteria and viruses. The infectious prion is thought to be an abnormally folded isoform ( $\text{PrP}^{\text{Sc}}$ ) of a host protein known as the prion protein ( $\text{PrP}^{\text{C}}$ ). The conversion of  $\text{PrP}^{\text{C}}$  to  $\text{PrP}^{\text{Sc}}$  occurs post-translationally and involves conformational change from a predominantly  $\alpha$ -helical protein to one rich in  $\beta$ -sheet structure.

**Alzheimer's disease** (AD) is the most common form of dementia in humans, of which the key pathological hallmark is the deposition of the 39-46 amino acid beta-amyloid peptide ( $\text{A}\beta$ ) in the brain.  $\text{A}\beta$  peptides are derived from sequential cleavage of the amyloid precursor protein (APP) by  $\beta$ - and  $\gamma$ -secretases.

Prion and Alzheimer's diseases involve the processing of distinct proteins which when misfolded cause neurodegeneration. Our laboratory is interested in understanding the molecular mechanisms by which these proteins exert their neurodegenerative properties and in the case of prion proteins, gain their infectious properties.

We are investigating the processing and secretion of the prion protein and the APP in association with small membrane vesicles known as exosomes. We have shown that exosomes can transfer infectious prions between different cell types.

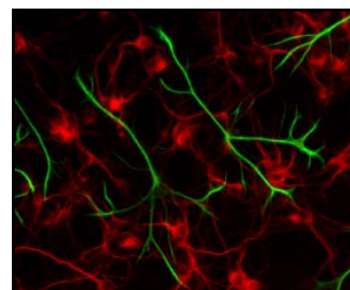


### Projects:

All projects will involve the use of various molecular biology techniques (eg, cloning, PCR, real time quantitative PCR, site-directed mutagenesis, next generation sequencing), cell biology (cell culture, immunofluorescent staining, live cell imaging, RNAi, transfection, lentivirus production, isolation of primary neuronal cells, prion infection assays), protein chemistry (recombinant protein expression and purification, protein gels, western immunoblotting, biophysical techniques), and general biochemical assays.

### How do highly conserved regions of PrP control prion formation?

Much remains to be understood about how the normal cellular isoform of the prion protein undergoes structural changes to become the disease associated form. We have developed several approaches to study this aspect of prion biology using cellular, molecular and biophysical techniques. We have refined a model



based around a highly conserved region of the prion protein (see publication 1 below). This project will use a combination of structural and cell biological approaches to investigate the mechanism of prion inhibition through this conserved region of the prion protein.

### Molecular mechanisms of Abeta toxicity in Alzheimer's disease - a role for the prion protein?

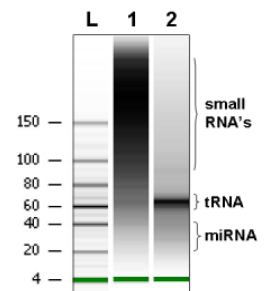
Recently it has been demonstrated that PrP can act as a receptor for A $\beta$  oligomers which are implicated in the pathogenesis of Alzheimer's disease. A $\beta$  is derived from a larger protein called the amyloid precursor protein (APP). We have well established cell lines expressing different forms of APP which generate A $\beta$ . These will be used to investigate the interactions of different forms of A $\beta$  with PrP using a combination of biochemical and cell biological assays to determine the molecular mechanisms underlying this interaction.

### Investigating the role of microRNA (miRNA) in neurodegenerative disorders such as Alzheimer's and Prion diseases (with Dr Shayne Bellingham)

This role of microRNA in regulating the expression of key genes and pathways will be investigated in both cell and animal models of neurodegenerative disease.

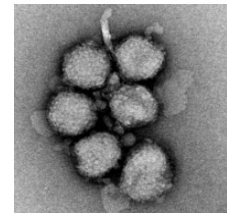
**Project 1.** Using our cell models we have identified a subset of microRNA genes that are enriched in exosomes and may regulate gene and protein expression by binding 3' UTR sequences in target cells. These novel sequences will be knockdown or over-expressed and tested for efficacy in treatment of neurodegenerative disorders. The project will involve techniques in cell culture, disease bioassays, qPCR, cell biology.

**Project 2.** The prediction of microRNA target genes is based upon computational analyses, the advent of next-generation sequencing technologies has allowed a new method called (CLIP)-seq assays to determine the exact microRNA sequences that bind to target genes. This project will determine the miRNA species that bind and regulate key genes involved in Alzheimer's and prion diseases.



### The role of the exosome pathway in processing of the prion and amyloid precursor proteins

This project will investigate the mechanisms, currently unknown, of exosome uptake by cells using modifiers of key cellular pathways and live cell imaging. This project will also investigate the role of modifiers in exosome biogenesis by altering their expression with RNAi, using a lentiviral delivery system in neuronal cells.



**Targeting the A $\beta$  peptide in Alzheimer's Disease Using an Intracellular Antibody** Supervisors: Dr Rebecca Nisbet (rebecca.nisbet@csiro.au); A/Prof Andy Hill; Dr Stewart Nuttall ([stewart.nuttall@csiro.au](mailto:stewart.nuttall@csiro.au)) Project Site: CSIRO Preventative Health Flagship, 343 Royal Pde, Parkville.

The aim of this project is to target A $\beta$  intracellularly prior to its aggregation. This will be done by transfecting mammalian cells with an A $\beta$  specific antibody that contains an endosome targeting signal peptide. Successful localisation of the intrabody and its ability to bind A $\beta$  and prevent its aggregation will initially be investigated, and intracellular A $\beta$  levels measured to assess intrabody-mediated clearance of A $\beta$ . This project is ideally suited to a candidate with interests in cell biology, antibody engineering and neurodegenerative diseases. Skills acquired will include diverse biochemistry and molecular biology techniques such as DNA cloning, protein and antibody expression and purification, and immunofluorescent microscopy.

#### Recent publications (selected)

1. Harrison CF, Lawson VA, Coleman BM, Kim YS, Masters CL, Cappai R, Barnham KJ, Hill AF. (2010) 'Conservation of a glycine rich region in the prion protein is required for uptake of prion infectivity', *J Biol Chem*, 285, 20213–20223.
2. Nisbet RM, Harrison CF, Lawson VA, Masters CL, Cappai R, Hill AF. (2010) 'Residues surrounding the GPI anchor attachment site of PrP modulate prion infection: insight from the resistance of rabbits to prion disease', *J Virol*. 84(13), 6678–6686.
3. Bellingham SA, Coleman LA, Masters CL, Camakaris J, Hill AF. (2008) 'Regulation of prion gene expression by transcription factors SP1 and MTF-1', *J Biol Chem*, Nov 6.
4. Wadsworth JD, Joiner S, Linehan JM, Desbruslais M, Fox K, Cooper S, Cronier S, Asante EA, Mead S, Brandner S, Hill AF, Collinge J. (2008) 'Kuru prions and sporadic CJD prions have equivalent transmission properties in transgenic and wild-type mice', *Proc Natl Acad Sci*, 105, 3885–3390.
5. Sharples RA, Vella LJ, Nisbet RM, Naylor R, Perez K, Barnham KJ, Masters CL, Hill AF. (2008) 'Inhibition of  $\gamma$ -secretase causes increased secretion of amyloid precursor protein C-terminal fragments in association with exosomes', *FASEB J*, 22, 1469–1478.
6. Vella LJ, Sharples RA, Lawson VA, Masters CL, Cappai R, Hill AF. (2007) 'Packaging of prions into exosomes is associated with a novel pathway of PrP processing', *J Pathol*, 211, 582–590.